

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Eva KONTEKOVA
Peter FILIPCIK

Serial No.: 10/521,049

Filed: November 1, 2005

For: TRANSGENIC ANIMAL EXPRESSING
ALZHEIMER'S TAU PROTEIN

Group Art Unit: 1633

Examiner: Leavitt, Maria Gomez

Atty. Dkt. No.: SONN:066US

Confirmation No.: 5434

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8I hereby certify that this transmittal is being electronically
filed with the United States Patent and Trademark Office
via EFS-Web on the date below:_____
January 9, 2008
Date_____
Travis M. WohlersTRANSMITTAL AND SUMMARY OF EXAMINER INTERVIEWCommissioner for Patents
P. O. Box 1450
Alexandria, VA 23313-1450

Sir:

Applicant submits herewith a copy of the 37 C.F.R. § 1.132 declaration of Dr. Filipcik ("Filipcik Declaration"). Applicant originally filed the executed Filipcik Declaration through the USPTO's Electronic Filing System on September 17, 2007. A subsequent Office Action mailed on November 28, 2007, indicated that the Filipcik Declaration had not been executed. Applicant reviewed the copy of the Filipcik Declaration on PAIR and discovered that the scanned document failed to reproduce Dr. Filipcik's signature and only faintly reproduced the date that Dr. Filipcik wrote next to his signature.

Upon learning of the scanning problem with the Filipcik Declaration, Applicant's representative contacted Examiner Leavitt to discuss the issue. In an interview on January 8, 2008, Examiner Leavitt advised Applicant's representative to resubmit the Filipcik Declaration along with an explanation that Applicant had originally submitted an executed declaration to the USPTO but the signature was not legible due to a problem with scanning the document. Examiner Leavitt stated that upon receiving the executed Filipcik Declaration the Office Action dated November 28, 2007 would be vacated and a new Office Action, which would consider the Filipcik Declaration, would be issued. Applicant would like to thank the Examiner for her assistance in addressing this issue.

The executed Filipcik Declaration submitted here is scanned from the identical document as the Filipcik Declaration executed on September 12, 2007, and filed on September 17, 2007. The contrast on the scanner has been adjusted to better reproduce Dr. Filipcik's signature.

The Examiner is invited to contact the undersigned attorney with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Travis M. Wohlers
Reg. No. 57,423
Attorney for Applicant

(Customer No. 32425)
FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
512.536.3035 (voice)
512.536.4598 (fax)

Date: January 9, 2008

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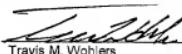
Examiner: Leavitt, Maria Gomez

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CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this declaration is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

September 17, 2007
DateTravis M. WohlersFILIPCIK DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Peter Filipcik, declare that:

1. I am a co-inventor of the above-referenced patent application. I am also an employee of Axon Neuroscience, the assignee of the above-referenced application. A copy of my *Curriculum Vitae* is attached as Exhibit 1.
2. It is my understanding that the Examiner in charge of the above-captioned application has advanced an enablement rejection against claims 17-33. I am supplying this declaration to provide additional evidence of the enablement of the present claims.
3. This declaration describes the generation of and studies on transgenic rat line #24. According to the teachings in the present specification, the DNA construct used for the

preparation of transgenic rat line #24 is characterized by the following features: (1) the cDNA molecule is truncated at least 30 nucleotides downstream of the start codon and truncated at least the 30 nucleotides upstream of the stop codon of the full-length tau cDNA sequence coding for 4-repeat and 3-repeat tau protein; (2) the cDNA molecule comprises SEQ ID NO:9; and (3) the DNA construct encodes a protein, which has neurofibrillary (NF) pathology producing activity when expressed in brain cells.

4. The transgene construct used in the generation of transgenic rat line #24 was prepared by ligation of a cDNA coding for human tau protein truncated at amino acid positions 93-302 into the mouse Thy-1 gene downstream of the brain promoter/enhancer sequence. This numbering is based on isoform 44 (3-repeat tau) as it is in the application. Amino acids 93-302 correspond to nucleotides 277-906, SEQ ID NO: 12 in the application. Thus, the truncated tau cDNA molecule used to generate rat line #24 is truncated at least 30 nucleotides downstream of the start codon and truncated at least the 30 nucleotides upstream of the stop codon of the full-length tau cDNA sequence coding for 4-repeat and 3-repeat tau protein; and the truncated tau cDNA molecule comprises SEQ ID NO: 9 (nucleotides 741-930). The truncated tau cDNA molecule also comprises SEQ ID NO: 12.
5. The cDNA coding for human tau protein in transgenic rat line #24 is shorter by 93 nucleotides (31 amino acids) than the cDNA coding for human tau protein in transgenic rat line #318.
6. The transgenic DNA was linearized by cleavage with EcoRI, and the vector sequences were removed prior to microinjection. Transgenic rats were generated by pronuclear injection of one-day old SHR rat embryos. Founders were screened by PCR using Thy-

1-specific and human tau-specific primers. Transgenic founder line #24, which stably expressed human truncated tau, was obtained.

7. Like transgenic rat line #318 described in the specification, transgenic rat line #24 exhibits neurofibrillary (NF) pathology. Transgenic rat line #24 developed neurofibrillary lesions in the brain stem, spinal cord, primary motor cortex, and hippocampus. Attached Figure 1 shows the staining of neurofibrillary lesions in the hippocampus and cortex of transgenic rat line #24 in the late stage of the disease.
8. Neurological examinations showed similar features in both the #24 and #318 transgenic rat lines. Sensory-motor impairment was measured by the "NeuroScale" method. NeuroScale represents a multi-test battery intended for the quantitative neurobehavioural evaluation of transgenic rats suffering from progressive sensorimotor neurodegeneration. Testing protocol enables complex sensorimotor, neuromuscular and neurological assessment of rats at different age periods. Complex neurobehavioural characterization of rats involves basic observational assessment, examination of neurological functions and evaluation of rat neuromuscular functions by prehensile traction test, assessing forelimb muscle strength and assessment of sensorimotor coordination abilities using beam walking test. This experimental strategy can reveal the impairment, which could otherwise be hidden and permits observation of changes caused by chronic neurodegenerative process. As shown in attached Figure 2, the progress of sensory-motor impairment of animals from transgenic line #318 and transgenic line #24 is almost identical. The onset and progression of neurodegeneration is the same in both transgenic rat lines. The transgene is transmitted to subsequent offspring generations and the phenotype stays unchanged even in the 4th generation of offspring.

9. Another measure of cognitive impairment is the object recognition test (ORT). ORT is used to measure object recognition memory, which is the ability to discriminate between objects that have been previously encountered and those that have not been. A spontaneous exploratory activity can be used for measurement of memory function in rats. ORT in animals is based on natural preference of investigating rather a novel than a familiar object. The intensity of memory storage can be tested using various types of delays between the first (presentation) and second (challenge) trial, in which the new object replaces a familiar object. As shown in attached Figure 3, transgenic rats from line #24 suffer from early cognitive impairment in the object recognition test.
10. The evidence discussed above demonstrates that transgenic rat line #24 contains a DNA construct having a cDNA molecule coding for N- and C-terminally truncated tau molecules having the following features: (1) the cDNA molecule is truncated at least 30 nucleotides downstream of the start codon and truncated at least the 30 nucleotides upstream of the stop codon of the full-length tau cDNA sequence coding for 4-repeat and 3-repeat tau protein; (2) the cDNA molecule comprises SEQ ID No. 9; and (3) the DNA construct encodes a protein, which has neurofibrillary (NF) pathology producing activity when expressed in brain cells.
11. I understand that the Examiner of this application asserts that there is no correlation between expression of any truncated tau protein in rat with any relevant characteristics or useful phenotype other than neurofibrillary pathology. This assertion is incorrect. First, neurofibrillary pathology is the most important and earliest immunohistochemical finding in Alzheimer's disease. Thus, an animal model that exhibits neurofibrillary pathology is a useful model of Alzheimer's disease. The transgenic rats described in the present

specification also exhibit other pathological features associated with Alzheimer's disease including cognitive impairment, oxidative stress, hypertension, and diabetes. As described in my declaration filed on January 10, 2007, transgenic rat line #318 exhibits cognitive impairment and oxidative stress (*see* para. 11). Additional studies conducted in my laboratory have also shown that transgenic rat line #318 exhibits hypertension – up to 220 mm/Hg compared to control rats at 121mm/Hg. Furthermore, diabetes can be induced in transgenic rat line #318 by using a specific high-carbohydrate diet. The spontaneous hypertensive rat strain is a genetic model for the study of obesity and diabetes. Obese rats exhibit both metabolic and histopathologic characteristics associated with non-insulin-dependent diabetes mellitus (type II) in humans. Obese male rats, when fed a high-carbohydrate diet, exhibit some of the metabolic alterations associated with human non-insulin-dependent diabetes mellitus, including hyperinsulinemia, hyperlipidemia, glucose intolerance, and glycosuria. Thus, the transgenic animals encompassed by the current claims are useful models of Alzheimer's disease because they exhibit the most important and earliest immunohistochemical finding in Alzheimer's disease (*i.e.*, neurofibrillary pathology) and they exhibit other pathological features associated with Alzheimer's disease including cognitive impairment, oxidative stress, hypertension, and diabetes.

12. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

12.9.2007
Date

Peter Filipcik

Peter Filipcik

Figure 1

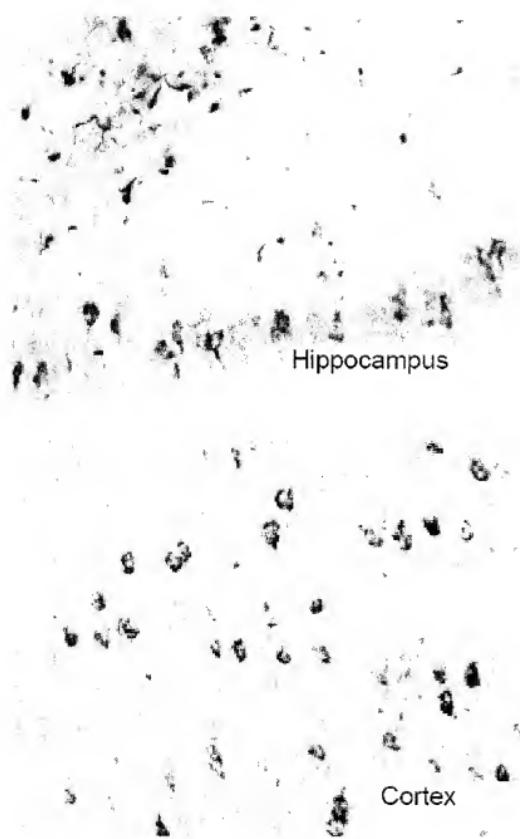


Figure 2

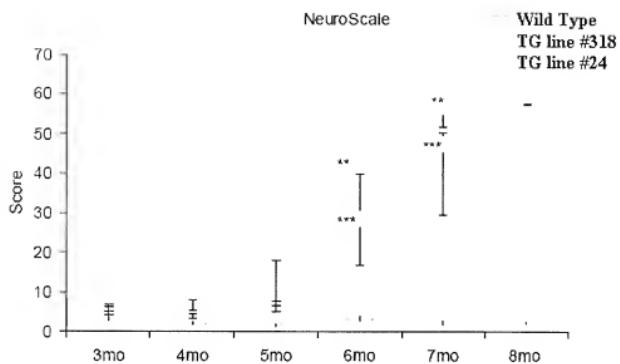
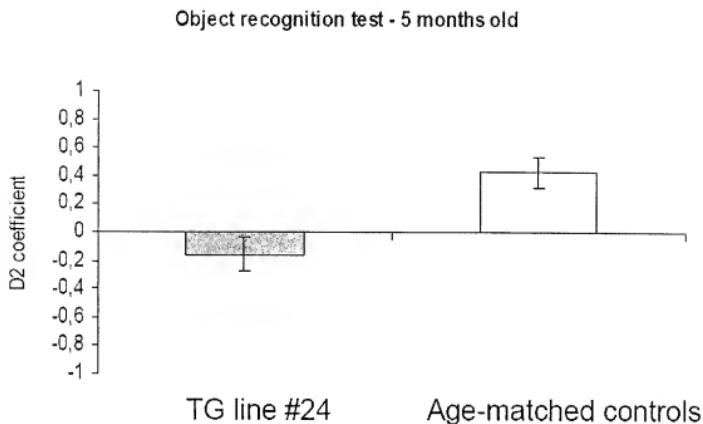


Figure 3



CURICULUM VITAE

NAME: RNDr. Peter Filipcik, PhD

BORN: June 26, 1962

CITIZENSHIP: Slovakia

ADDRESS: Podhaj 3, Lamac, 84103 Bratislava,
e-mail: peter.filipcik@savba.sk

EDUCATION:

June 1985 PhD Slovak Academy of Sciences, Bratislava, Slovakia

June 1986 RNDr. Comenius University, Faculty of Natural Sciences in Bratislava, Slovakia

EMPLOYMENT:

1996 – pres Senior scientist - Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia (part time)

2001 – pres Senior scientist - Axon Neuroscience GmbH, Vienna, Austria

1986 - 1996 Research assistant, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

2000 - 2001 University of Vienna, Vienna, Austria

1998 - 2000 Visiting scientist at the CCRI, St. Anna Children Hospital, Vienna, Austria

1995 - 1996 Research associate, Dept. of Pharmacol., University of Minnesota, Minneapolis, USA

1993 - 1994 Research assistant, Dept. of Chem. Pharmacol., University of Tokyo, Japan

INTERNATIONAL COURSES AND MEETINGS ATTENDED (selection):

1990 "3rd European Congress on Cell Biology", Florence, Italy

1993 "The Radioisotopes in Biological Research", The Univ. of Tokyo, Tokyo, Japan

1993 "5th Inter-Department Meeting on Chemical Pharmacol.", Seoul, South Korea

1998 "6th Int. Conf. on Alzheimer's Disease and Related Disorders, Amsterdam, Netherlands

2001 "Ageing and Dementia - Current and future concepts", Graz, Austria

2003 In Vitro Human Cell Systems Enabling Drug Discovery , London, UK

2004 "9th International Conference on Alzheimers Disease and Related Disorders", Philadelphia, Pennsylvania

2005 Molecular Medicine Triconference, CHI, San Francisco, California, USA

2006 "10th International Conference on Alzheimers Disease", Madrid, Spain

MEMBERSHIP OF LEARNED SOCIETIES:

1997 Slovak Immunological Society

1996 The Slovak Alzheimer Society

2005 The Slovak Neuroscience Society

PUBLICATION ACTIVITY:

Author and co-author of 21 scientific papers, 2 patents

Bratislava 6. 12. 2006

List of publications:

Filipcik P, Cente M, Ferencik M, Hulin I, Novak M. The role of oxidative stress in the pathogenesis of Alzheimer's disease. *Bratisl Lek Listy*. 2006; 107 (9-10), 384-394

Pevalova M, Filipcik P, Novak M, Avila J, Iqbal K. Post-translational modifications of tau protein. *Bratisl Lek Listy* 2006; 107 (9-10), 346-353

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Filipcik P, Strbak V, Brtko J. Thyroid hormone receptor occupancy and biological effects of 3,5,3,-L-triiodothyronine (T3) in GH4C1 rat pituitary tumour cells. *Physiol Res*. 1998;47(1):41-6.

Wei LN, Lee CH, Filipcik P, Chang L. Regulation of the mouse cellular retinoic acid-binding protein-I gene by thyroid hormone and retinoids in transgenic mouse embryos and P19 cells. *J Endocrinol*. 1997 Oct;135(1):35-46.

Nikodemova M, Weismann P, Filipcik P, Mraz P, Greer MA, Strbak V. Both iso- and hyperosmotic ethanol stimulate release of hypothalamic thyrotropin-releasing hormone despite opposite effect on neuron volume. *Neuroscience*. 1997 Oct;80(4):1263-9.

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*Equal contribution.

NEUROBIOLOGY OF AGING supplement:

Filipcik, P; Pevalova, M; Smrzka, O; Novak, M. Neuronal assay based on developmentally inducible expression of Alzheimer's tau, designed for screening of AD therapeutics. *NEUROBIOLOGY OF AGING*, JUL 2004, 25, Suppl. 2, S265

Pevalova, M; Filipcik, P; Mederlyova, A; Cente, M; Smrzka, O; Novak, M Hyperphosphorylation and oxidative stress as early changes in axon's new AD rat model. *NEUROBIOLOGY OF AGING*, JUL 2004, 25, Suppl. 2, S264

Cente, M; Filipcik, P; Hanusovska, E; Zilka, N; Novak, M Onset and intensity of AD changes in transgenic rat expressing Alzheimer specific Tau protein correlates with gene dosage. *NEUROBIOLOGY OF AGING*, JUL 2004, 25 Suppl. 2, S239

Hrnkova, M; Zilka, N; Filipcik, P; Novak, M Cognitive deficit and progressive motor impairment in AD rat model. *NEUROBIOLOGY OF AGING*, JUL 2004, 25, Suppl. 2, S233

Koson, P; Zilka, N; Filipcik, P; Novak, M Neuronal loss in selected brain areas of a new transgenic AD rat model estimated with unbiased stereological methods, *NEUROBIOLOGY OF AGING*, JUL 2004, 25 Suppl. 2, S249, S250.

Zilka, N; Csokova, N; Vechterova, L; Skrabanova, M; Hrnkova, M; Filipcik, P; Novak, M. Staging of neuropathological changes in axon's novel transgenic AD rat model is linked to a lethal phenotype. *NEUROBIOLOGY OF AGING*, JUL 2004, 25, Suppl. 2, S255